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Metabolomics, Physical Activity, Exercise and Health: a Review of the Current Evidence

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KEYWORDS:

Physical Activity; Exercise; Metabolomics; The Metabolome; Health; Health Promotion;

HIGHLIGHTS:

- Physical inactivity is a strong disease risk factor, but mechanisms are elusive.
- Metabolomics is ideally suited to explore the physical activity-health relationship.
- Literature supports metabolomic response to physical activity and exercise that can differ between individuals.
- Volume of physical activity and exercise may be the biggest drivers of the metabolomic adaptations.
- Branched chain amino acids, Tricarboxylic Acid (TCA) cycle, oxidative stress and fatty acid mobilization are key.

ABSTRACT

Physical activity (PA) and exercise are among the most important determinants of health. However, PA is a complex and heterogeneous behavior and the biological mechanisms through which it impacts individuals and populations in different ways are not well understood. Genetics and environment likely play pivotal roles but further work is needed to understand their relative contributions and how they may be mediated. Metabolomics offers a promising approach to explore these relationships.

In this review, we provide a comprehensive appraisal of the PA-metabolomics literature to date. This overwhelmingly supports the hypothesis of a metabolomic response to PA, which can differ between groups and individuals. It also suggests a biological gradient in this response based on PA intensity, with some evidence for global longer-term changes in the metabolome of highly active individuals. However, many questions remain and we conclude by highlighting future critical research avenues to help elucidate the role of PA in the maintenance of health and the development of disease.

Physical Activity and Health

Physical inactivity is a worldwide health problem, and is ranked as the fourth leading behavioral risk factor for global mortality [1]. The World Health Organization (WHO) recommends adults undertake at least 150 minutes moderate or 75 minutes vigorous intensity physical activity (PA) per week [2], conduct muscle strengthening activities twice a week, and minimize time spent being sedentary, with similar guidelines for under 18s and older adults. Regular PA can reduce the risk of over 20 chronic conditions including coronary heart disease, stroke, type 2 diabetes, some cancers, obesity, mental health problems such as depression, and certain neurological conditions including dementia. Importantly, as well as reducing disease risk, it can also promote well-being and quality of life [3]. Consequently, harnessing the power of PA could have far-reaching preventive public health impacts. However, to do so is complicated by the fact that PA is not a single behavior but rather a complex and heterogeneous set of actions. It can be classified by domain (occupational, leisure-time, travel, home), dimension (frequency, intensity, duration), context (access to greenspace, facilities, and resources like money and social support), motivation (reasons for undertaking PA) and type (which can range from aerobic treadmill exercise through yoga to housework and gardening) [4].

As with many areas in population health the associations between risk (low PA) and health are well documented but the mechanisms involved and their complex interactions are not yet fully elucidated [5]. Despite growing epidemiological evidence for a relationship, how different dimensions and types of PA, and the domains and contexts in which they are undertaken, impact the health of individuals and populations in different ways is not well understood. Similarly, the reasons and mechanisms explaining why the same PA behaviors appear to have differential impacts within a population remain elusive. Genetics and environment likely play pivotal roles but further work is needed to understand their relative contributions and how they may be mediated [6]. Metabolomics offers a promising approach to explore these relationships.

Metabolomics

Metabolomics is the systematic study of all the metabolites (i.e. small molecules <10kDa, including carbohydrates, amino acids, organic acids, nucleotides and lipids) in a biological sample utilizing either Mass-Spectrometry (MS) or Nuclear Magnetic Resonance Spectroscopy

(NMR) [7]. It provides a means of characterizing metabolic differences between groups and individuals, while informing understanding of the biology and biological mechanisms underlying these differences. The metabolome reflects the genome, the transcriptome and the proteome as well as their interactions with the environment. In this way, it is a means of studying the life course experiences of an individual [8]. The metabolome can capture past exposures (both short and longer term), indicate likelihood of future phenotypes and reflect current-status and responses [7]. As such, metabolomics is ideally suited to the study of PA. Its potential role in this field is evidenced, both by the increasing body of literature as well as by initiatives such as MoTrPAC [9] (The Molecular Transducers of Physical Activity Consortium, <https://www.motrpac.org>). This is a nationwide US research consortium funded by the US National Institutes of Health, that aims to study molecular, including metabolomic, changes relating to PA to improve the understanding of how PA influences health [10].

Widespread alterations in metabolite levels are known to occur when a biological system is in a dysregulated or exacerbated state. Accordingly, changes in components of the metabolome induced by intense and strenuous exercise regimes have been noted for many decades [11, 12]. In fact, as early as 1975, Wahren et al *et al.* [13] argued that the metabolic status following 40-60 minutes of upright continuous bicycle exercise can be comparable to a few days starvation, with changes relating to gluconeogenesis, increased protein catabolism, increased urea excretion and the metabolism of nitrogenous substances [12, 13]. Despite this, PA is known to induce numerous positive short and long health effects and can lead to beneficial long-term physiological adaptations [14].

This narrative review describes currently published literature on the metabolomics of PA (and exercise) in humans. Our aim is to understand the breadth and depth of the current evidence base, expanding upon and updating previous reviews in this area [15-17]. We highlight current understanding and potential novel research priorities by mapping the themes in the literature base, which continues to grow rapidly. Critical analysis of this literature will afford an improved understanding of the status of PA metabolomics, help to identify critical evidence gaps, and inform future studies [18].

The metabolomics of PA literature falls into two general groups based on their aims and study designs and populations: (i) observational studies that compare the average PA behaviors or overall fitness levels (a well-established outcome of PA), as assessed via questionnaire, fitness

testing or participation in certain elite sports, and the corresponding metabolomes between groups (**Table 1**); and, (ii) experimental intervention studies that measure metabolites before, after and/or during PA or exercise interventions. The intervention trials can be further subdivided into two categories; (ii.a) short-term interventions that focus on a discrete bout or set of bouts of PA/exercise within short time frame (< 1 week) (**Table 2**) and (ii.b) long-term interventions that assign individuals to training or PA/exercise interventions lasting more than 1 week and compare individuals before and after this training (**Table 3**). For both short- and long-term interventions, a majority of studies compare the same individuals pre- and post, while the remainder compare different groups subject to the same or to differing interventions. There are also subsets of the literature that focus specifically on “at-risk” populations including overweight/obese individuals and those with certain diseases, and those that focus on elite athletes.

A brief methods description is provided in **Supplementary Methods**, and the included studies are described in **Tables 1 2 and 3**. When reviewing the literature, “Physical activity” and “exercise” are important terms to distinguish. As mentioned above, exercise is a sub-type of PA generally more structured and repetitive, and more strongly associated with health and fitness [19]. Laboratory studies employing treadmills, ergometers, etc. are more likely to be using “exercise” as their independent variable. However, exercise has been shown to be a small contributor to overall population PA levels [20, 21], and as such differentiating is important to understanding the implications of PA metabolomics evidence. We found that the terms PA and exercise have been variously and inconsistently defined throughout these studies, and at times used synonymously, although the majority of studies focus on structured exercise. As such we have used the term “PA/exercise” where appropriate so as not to miss-represent the evidence base, and where necessary we have employed the wording used in the cited study. Despite these inconsistencies in definitions, there are a number of overarching themes that are consistent throughout the existing literature as we report below.

Table 1: Observational Studies Included in This Review

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Table 2: Short-term Intervention Studies Included in this Review

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Table 3: Long-term Intervention Studies Included in this Review

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The PA/Exercise Metabolome

The literature to date, with some notable exceptions [22-25], overwhelmingly concludes that varying degrees of PA or exercise induce, or are associated with, quantifiable changes in the metabolome, as measured by MS and NMR spectroscopy in biological samples [15-17]. The metabolomic alterations associated with overall PA/exercise are most commonly characterized by changes in fatty acid metabolism, mobilization and lipolysis, the TCA cycle, glycolysis, amino acid metabolism, carnitine metabolism, purine metabolism, cholesterol metabolism and insulin sensitivity among others [26-69] (**Figure 1**). These findings broadly reflect the known physiological adaptations to PA/exercise. The reported pathways and metabolites are inextricably linked through their roles in increased anaerobic and aerobic metabolism, increased Adenosine Triphosphate (ATP) turnover, changes in substrate utilization energy metabolism, lipolysis, fatty acid oxidation, ketogenesis, branched-chain amino acid catabolism, glutathione metabolism and oxidative stress [31, 37, 44, 47, 60, 62, 70].

Figure 1: Metabolites, Metabolomics Pathways and Biological Processes Reported in the Literature for Different Categories of Physical Activity Interventions

Metabolites and pathways are colored according to Metabolon Superpathway [71] where available, or else are denoted according to their biological process

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There is some debate in the literature as to which components of PA/exercise lead to the most pronounced changes. Some of the strongest reported findings related to the most “intense exercise”, as defined by variously by kJ expenditure, VO₂ peak and type [38, 39, 51, 53, 61], and among those studies that did not see a PA/exercise associated change in the metabolome, Zheng *et al.* (2014), postulated that this may be due to low intensity levels in their study [22]. However, a number of other studies considering only moderate and low intensity PA/exercise have reported significant findings, and those studies directly comparing metabolomic patterns associated with light, moderate and vigorous PA/exercise patterns generally conclude that while the metabolomic

patterns may differ slightly between these groups, overall volume was the biggest driver of the metabolomic profile [27, 39, 41, 72]. This points to a multifactorial dose response relationship between PA/exercise and its effect on the metabolome, incorporating intensity, bout length, frequency and timeframe of exposure (hours, days, weeks, years).

Acute versus long-term effects of PA/exercise on the metabolome

The evidence therefore suggests PA/exercise affects the metabolome, but the question of when these changes occur and for how long they persist, is more complex. However, it is crucial to understand the effects of long-term and sustained PA/exercise as they may have important implications for weight loss or training and for public health more generally. It has been reported in several studies that PA/exercise associated metabolic alterations were evident almost as soon as the activity begins [15, 34, 39]. However, the studies differed in their findings during the post-activity and recovery phases; some reported a return to a pre-activity metabolic state almost immediately (within minutes) while others reported the effects of acute PA/exercise was evident in the levels of some metabolites for hours [31, 34, 35, 67] or even days [54, 66]. Importantly, it was also noted that the timing of the change in metabolite levels and how long they persisted differed depending on the length and intensity of the exertion [35].

Differences were further noted between metabolites and metabolite classes [35]. For example, metabolic pathways responsible for skeletal muscle substrate utilization were shown to be changed immediately after “acute exercise”, while changes in metabolites of the TCA cycle were apparent 60 minutes later [31, 55]. These differences likely reflect the timing of the physiological adaptations to the onset of the activity and the metabolites corresponding to this physiology. Amino acids including leucine, isoleucine, asparagine, methionine, lysine, glutamine, alanine and lipids have been reported to remain significantly decreased after 14 hours of post-activity recovery while changes in plasma medium- and long-chain fatty acids, ketone bodies, bile acids and triacylglycerol esters, have also been shown to persist for many hours after activity [35], before eventually returning to pre-activity levels [15].

While the literature undoubtedly supports the existence of acute metabolomic effects of PA/exercise, many critical public health outcomes manifest over timeframes of weeks, months, years and decades. Therefore, it is important to also study and understand the longer term

metabolomic and health changes associated with PA/exercise. Observational studies provide further insights into these longer-term effects by directly comparing groups of individuals with differing levels of previous exposure in a cross-sectional manner to determine whether their metabolomes differed (**Figure 2**) [46, 70, 72-76]. Overall, the findings we present in this paper suggest that there may also be a chronic adaptation in the metabolome in response to a long-term PA/exercise regime that is characterized by changes in lipids and amino acids. A large study of men in Japan that used self-reported questionnaires to assess metabolomic changes with long-term PA, observed lower levels of amino acids and higher levels of pipercolate in more active men [70], while a study of over 5000 individuals from four cohort studies replicated a number of long term PA associated metabolites including several lipids [76]. However, the underlying metabolic variability between individuals due to the inherently dynamic nature of the metabolome can render the comparison of groups based on a single metric complex, particularly when the actual changes in metabolite levels have been shown to be small [75]. To overcome this, Kujala *et al.* compared sets of persistently discordantly active twins over multiple decades. They determined that with persistent PA over time the fatty acid composition of the metabolome shifted from a saturated to a more polyunsaturated profile, with lower levels of glucose, and isoleucine among the active [73]. Importantly, they were able to validate these findings in an independent population-based cohort. Among the intervention studies, while some studies comparing metabolomic profiles of individuals before and after a long-term or PA/exercise intervention observed a clear distinction in the pre- and post- metabolomics profiles [36, 43, 66, 68], several others have reported no differences [22-25, 69]. Consequently, further work is needed to disentangle whether long-term PA/exercise can shift the metabolome, and by how much.

Figure 2: Metabolites, Metabolomics Pathways and Biological Processes Reported in the Literature in Observation PA studies

Metabolites and pathways are colored according to Metabolon Superpathway [71] where available, or else are denoted according to their biological process

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With the assumption that there are metabolic differences between persistently physically active versus persistently inactive people, several studies have suggested these differences may be even greater when undertaking PA/exercise, than when at rest [29, 32, 34, 36]. This indicates that the adaptations in the metabolome induced by a PA/exercise regime may continue to influence an individual's response to acute activity [29, 56]. Interestingly, Schader *et al.* noted that among men

running a marathon, it is the slower, less aerobically fit runners who experience the greatest perturbation in metabolite levels, specifically phospholipids and amino acids [54]. The hypothesis of a possible metabolomic adaption to PA/exercise is further supported by evidence that metabolic signatures of “exercise-trained” skeletal muscle reflected reprogramming of mitochondrial function and intermediary metabolism [67]. However, not all studies are in agreement with it being argued by some that “associations of current activity with most metabolic traits do not differ by previous activity” [75].

A subset of the literature is specifically focused on elite athletes, in whom it may be assumed that any metabolomic adaptations would be most pronounced. It is well known that the physiology of athletes differs from that of the normal population in a variety of ways. Depending on the type of athlete these may include greater heart function (e.g. stroke volume, left ventricular volume), higher maximal oxygen utilization (VO₂ max), greater lung volume, lower body fat percentage and differing fiber-type compositions of the muscles [16]. What the metabolomics literature now indicates, is that athletes may also differ in terms of an altered metabolome (**Figure 2**). In particular, differing levels of lactate, β -D-methylglucopyranoside, pyroglutamic acid, free fatty acids, amino acids, carnitines, trimethylamine-N-oxide (TMAO), and carboxylic acid derivatives, among others, have all been observed between athletes and untrained individuals. Taken together this points to underlying differences in intermediary metabolism, fuel substrate utilization, glucose transport, fatty acid oxidation, oxidative stress, steroid biosynthesis, insulin signaling and microbial density between these groups [26, 27, 55, 56, 58].

There is also a body of literature investigating whether the type of PA/exercise influences the metabolomic response. Metabolomic differences between sporting groups, including athletics, boxing, cycling, football, rowing, rugby and swimming, have been reported [27]. One study identified differences between the goal keeper and the field players participating in the same game [65]. Additional differences have been noted between athletes competing in dynamic (e.g. marathon running) as opposed to static sports (e.g. weightlifting) and those competing in sports considered to be both dynamic and static such as rowing or boxing [27]. In particular, elevated levels of metabolites involved in sex steroid hormone biosynthesis and rapid changes in whole-body substrate utilization characterized by upregulated lipolysis, fatty acid oxidation, amino acid oxidation, ketogenesis, and glycolysis have been observed [27, 30, 35, 44]. Again, these differences are most likely explained by the intensity as a function of the effort, strain and duration of a given activity rather than the protocol itself [38, 41, 53, 66]. This would provide a

rationale for the finding of an increased augmentation of pantothenate and fumarate in individuals who had just completed a marathon, which were not observed after a short-term treadmill intervention, as well as metabolomic differences between faster versus slower marathon runners [31]. Consequently, it has been argued that when comparing different training modalities, it is vital to match on overall effort [41].

Metabolomics and fitness

It should further be noted that, some of these differences may be related to pre-existing fitness levels rather than the PA/exercise dose. As well as differences in age, and geography between the cohorts, there are strong socioeconomic differences in participation in different sports that could (at least in part) explain metabolomic differences [77]. Similarly, some sports may require very specific dietary regimes that impact the metabolome, while others such as weight-lifting, may both promote specific diets as well as select for people with a certain underlying physiology or phenotype. Furthermore, it has been shown that athletes from different sports may exhibit a distinct xenobiotic profile (i.e. exogenous metabolites not naturally produced in the human body) reflecting their drug/supplement use, diet and exposure to various chemicals, such as Trichloramine in swimmers [62] or grass exposure in footballers [78]. Baseline levels of metabolites, BMI and even the altitude at which the exercise is undertaken may also influence the metabolomic response [31, 45, 55]. All of these factors further complicate the comparisons of the metabolomic response to different PA/sport/exercise regimes. In addition, there is increasing evidence that social stimuli during PA/exercise can influence health outcomes due to psychosocial processes [79] as well as social cohesion and adherence [80].

The study of the metabolomic response to PA/exercise is, therefore, inextricably linked to the ‘metabolome of fitness’ (by which we mean exercise related fitness such as aerobic fitness, muscle strength, muscle endurance, and flexibility). Indeed, it has been reported that cardiorespiratory fitness is more strongly correlated with metabolic networks than with measures of PA/exercise [23, 74]. A study by Morris *et al.* noted that individuals could be distinguished into fitness levels on the basis of their metabolome [46], while Castro *et al.* similarly reported a dose-response correlation between cardiorespiratory fitness and the metabolome [51]. As with the findings observed in the elite athletes, overall, fitter individuals (as defined by maximal oxygen consumption) have been shown to have lower levels of amino acids particularly branched chain amino acids, likely due to increased gluconeogenesis, more efficient utilization of fat stores [46,

74], and a metabolomic profile that enables a greater capacity to activate lipolysis, facilitate entry of fatty acids into the TCA cycle, and to expand the TCA cycle intermediate pool during PA/exercise [31]. The antioxidant and cardiorespiratory inflammatory defense systems have also been shown to improve with exercise training. Perhaps not unsurprisingly lower levels of amino acids have also been associated with improved “post-exercise” recovery times [55]. Recovery is a critically important component of PA/exercise, and a number of studies have specifically explored this aspect. The results suggest that recovery may also exhibit unique metabolomic changes between individuals [52] that again depend on baseline metabolic profile [55], and which according to one study, may be even greater than the changes associated with the exercise itself [39].

Beneficial health effects

One of the primary reasons that individuals undertake many physical activities is for the purported health benefits. Based on the unique properties of the metabolome as the “ome” closest to phenotype and the ability to inform on past exposures, it can therefore be hypothesized that the beneficial health effects of PA/exercise can be tracked via the metabolic alterations it induces. Indeed, evidence suggests higher overall volume of habitual PA/exercise is associated with metabolomic signatures that are consistent with better cardiometabolic health [72], and physically active individuals have a “coherently healthier metabolic profile than their inactive counterparts” that helps to reduce the increased cardiometabolic risk associated with a sedentary/inactive lifestyle [73]. Although it should be noted here being active and having high levels of sedentary behavior are not mutually exclusive and there is some debate in the literature regarding the different risks and benefits attached to these two states [81].

The “coherently healthier metabolic profile” has been variously defined as lower levels of isoleucine, α 1-acid glycoprotein, VLDL cholesterol, triglycerides and glucose, an overall less saturated fatty acid profile and a shift towards large high-density lipoproteins [26, 72, 75]. The impact of PA/exercise on amino acids, particularly branched chain amino acids, has been suggested to result from the activation of key enzymes involved in BCAA degradation, and these lower levels reflect an increase in fat oxidation efficient utilization of fat stores, more efficient lipid metabolism and fatty acid oxidation and more efficient adjustment of energy expenditure during recovery which may lead to improved insulin sensitivity [30, 46, 53, 64, 72]. It has further

been suggested that that high fitness status induces an increased cardiorespiratory inflammatory and antioxidant defense system, more prone to deal with the inflammatory response following PA/exercise [31, 33, 62]. Metabolomics can also help understand the potential negative effects of strenuous PA/exercise, including elucidating the mechanism underlying disorders induced by strength-endurance activities, such as overtraining and fatigue [52, 56, 59].

Interestingly, it has been observed in a number of studies focusing on diseased populations that although the majority of PA/exercise-associated metabolic changes are the same in all people, including alterations in lactic acid, hypoxanthine, inosine and alanine levels, which were identified in multiple studies [23, 31, 33, 40-42, 48, 50, 51, 53, 55-58, 60, 61, 63, 65, 75], the metabolomic response can differ between healthy and diseased population in a metabolite specific manner [28, 31, 62]. This leads to the question as to whether PA/exercise has a differential impact on the metabolome in those who are susceptible to disease, or whether the disease associated metabolomic changes outweigh those due to PA/exercise. Additional work in more diverse populations, with a focus on the magnitude of absolute changes in metabolite levels, is required to disentangle this further.

Obesity and weight management

A number of studies have been conducted in populations that included individuals who were overweight or obese [22, 23, 66-69]. Obesity and overweight are whole system disorders known to elicit a coordinated metabolic response [82, 83], and therefore to impact the metabolome. There is some contention regarding the role of “exercise” in BMI and weight management [84]. However, the underlying metabolomic hypothesis is that PA/exercise induces AMP-activated protein kinase pathway in muscle and other tissues, which increases fat oxidation and glucose transport [83], and additionally acts upon insulin sensitivity to increase fat loss. Given the involvement of these pathways, changes in weight will also likely influence the metabolome. Therefore, the interconnected relationships between PA/exercise, weight, weight changes and the metabolome, complicate the interpretation of metabolomics studies of PA/exercise that are specifically targeted at weight loss. It may be hard to disentangle those metabolomic changes that are due to a decrease in BMI, from those that are due to the PA/exercise that precipitated that change in BMI. Indeed, many metabolites previously reported in the literature to be associated with BMI, were also identified by studies in this review as associated with PA/exercise, including

branched-chain and aromatic amino acids, long-chain polyunsaturated fatty acids, phospholipids, lysophosphatidylcholines, sphingomyelins, carbohydrates, nucleotides and carnitines [27, 29, 30, 34, 36, 40, 42, 43, 47, 53, 54, 60, 67-70, 85].

PA/exercise has been shown in one study to influence the metabolome independently of weight loss. Meucci *et al.* tracked the urinary metabolome across an eight-week exercise intervention. Although this intervention resulted in a 7% decrease in body fat that did induce metabolic changes, they were also able to identify independent metabolic alterations that related to the activity itself [68]. However, it should also be noted that, three other studies of medium to long term PA/exercise interventions in overweight/obese individuals, observed no change in the metabolome at all [22, 23, 69]. Furthermore Brennan *et al.* noted that the levels of certain metabolites did change with a change in adipose tissue deposits, but that these changes could not be attributed to the long-term PA/exercise intervention [25]. Consequently, further work is necessary to determine the role of BMI in the PA/exercise-metabolomics relationship.

Sex and PA/exercise metabolomics

Several metabolomics studies have shown baseline differences in the metabolome of males and females [86]. Similarly, due to hormonal and muscle mass differences between the sexes, men and women tend to show differences in their physiological responses and adaptations to PA/exercise [87]. Therefore, it can be hypothesized that they may also differ in their metabolic response to PA/exercise, however, there is some disagreement in the literature as to whether this is actually the case. Sex differences in the metabolomic responses to activity in the literature have been noted, with differential directions of effect for certain metabolites [27] and metabolite sex interactions for others [46]. In this latter study, the beneficial effects of PA/exercise, including more efficient utilization of fat stores during PA/exercise, more efficient adjustment of energy expenditure during recovery, and improved insulin sensitivity were shown to be most pronounced in females [46]. This may result from the fact that females are likely to utilize more fat and less protein as an energy source during PA/exercise [22]. Similarly, it has been reported that the observed metabolite-PA/exercise energy expenditure associations, including for valine, alpha-hydroxyisovalerate, 2-hydroxybutyrate (AHB), mannose, glucose and threonate were stronger in men than in women, and additionally that some metabolites were associated with

PA/exercise energy expenditure in women only [72]. However, often where any differences were reported, the direction of effect was the same between both sexes, it was just the magnitude of effect that differed slightly [46, 72], which may be a function of power and sample size rather than biology.

Furthermore, the studies that did report differences are relatively small, and it should be noted that a number of other studies have reported no differences by sex in the response to the same intervention or sex-specific differences in the PA/exercise associated metabolome [35, 76]. Additionally, among the studies selected for this review there is no evidence of a systematic difference in the results between the all-male and the all-female populations. Perhaps even more importantly, a large number of studies have either not reported sex specific results at all or have only included men in their study populations.

Biological sample

A common criticism of metabolomics studies is that they often profile blood or urine rather than the tissue most proximal or relevant to the outcome of interest. For PA/exercise, it is debatable which tissues this would be. Skeletal muscle is one of the most important organs in terms of the metabolic adaptations accompanying PA/exercise. Consequently, it is here that you may expect to see some of the greatest metabolic changes. Indeed, Huffman reported that the metabolic signatures of “exercise-trained skeletal muscle” reflected reprogramming of mitochondrial function, intermediary metabolism and whole body insulin sensitivity, which in turn augments oxidative capacity and “exercise tolerance” [67]. Nevertheless, blood is the most commonly used biologic media reported in the existing literature, with the remainder comprising a combination of muscle, urine, exhaled air, breath condensate, saliva and feces. [22, 26, 32, 34, 40, 46, 51, 57-63, 65, 67, 68]

There is some inconsistency in the literature regarding whether the same metabolomic changes can be observed in different tissue types. Many studies report similar results regardless of biosample, including plasma, urine, exhaled air, breath condensate, saliva and stool [26, 34, 46, 65], and in blood samples from both “exercising and non-exercising” parts of the body [31]. Conversely, in a comparison of urine and stool, Barton *et al.* observed that the metabolomic

differences between elite rugby players and controls was greatest in the urine [26], while Castro *et al.* also noted some difference between serum and skeletal muscle [51].

Other Omics

It is increasingly recognized that metabolomic data alone may be insufficient to fully characterize complex pathologies [88]. The metabolome represents the most downstream ‘ome’, therefore it is also of biological interest to characterize the upstream ‘omes (i.e. the genome, epigenome, transcriptome and proteome) that may play a role in driving the metabolic changes. The integration of metabolomics with other omics data to identify the interactions and synergies between the different hierarchical components of the ‘central biological dogma’ represents a potential strategy that will allow the visualization of a biological system on the most global level [6]. Accordingly, several studies [26, 31, 58, 67] also reported additional omics data or integrative omics analyses.

Overall these results provided a measure of validation of the metabolomics findings. In general, alterations in metabolite levels were accompanied by an up or down regulation of the gene sets encoding the enzymes catalyzing the relevant metabolome reactions e.g. acylcarnitines with lipid metabolic genes involved in muscle uptake and oxidation of fatty acids and succinate with genes involved in oxidative phosphorylation [58, 67]. Similarly, upregulation of *nur77* a transcriptional regulator of glucose utilization and lipid metabolism genes in skeletal muscle was shown to be driven by a combination of glycerol, niacinamide, glucose-6-phosphate, pantothenate, and succinate that increased in plasma in response PA/exercise [31]. The microbiome has also been explored in relation to the metabolome of PA/exercise. Again, the additional omic results expanded upon and provided a further rationale for the observed metabolomics findings, with observed increases in both metabolites and microbiota associated with enhanced muscle turnover [26].

Discussion

The associations between PA/exercise and health are well described in the epidemiological literature [2] as are the physiological processes of adaptation to PA/exercise [89]. However, the

metabolomic biology underlying the differential effects within and between individuals in terms of improvements in fitness and in health is less well understood. Our review has demonstrated that PA-metabolomics has the potential to address some of the biggest mechanistic questions in the study of PA, exercise and health. This includes the metabolic adaptations that occur in response to PA/exercise, and crucially how these adaptations may influence the maintenance of health, the development of disease, the regulation of a healthy weight, rehabilitation or other physical and psychological health related outcomes. Furthermore, it also provides the potential for the identification of clinically useful biomarkers.

The metabolomics literature to date overwhelmingly supports the hypothesis of a metabolomic response to PA/exercise, and a differential response between individuals. It also suggests a biological gradient in this response based on the duration or intensity of the activity, with some potential evidence for global longer-term changes in the metabolome of highly active individuals. Several common themes emerged throughout the studies relating to, branched chain amino acids, the TCA cycle glycolysis, oxidative stress, insulin sensitivity and fatty acid mobilization. As well as specific metabolites such as glucose, pyruvate, succinate and alanine, serine, glutamate, sarcosine, carnitines and kynurenine.

Nevertheless, there is substantial heterogeneity in the PA metabolomics literature, in terms of PA/exercise exposure and how it is measured, study design, intervention characteristics, sample type, timing of collection and the underlying study population. The results from the literature to date suggest this latter point may be of particular importance, and as such it is unclear to what extent differences in results between studies reflect true biology and to what extent they are merely a function of the heterogeneity between the studies, including sex, age, geography, fitness level, nutrition and disease status. The vast majority of studies have been conducted in small populations, making it difficult to further interrogate these questions with the data available. Of the 54 studies detailed in **Tables 1, 2 and 3**, 43 (80%) included less than 100 participants and 31 (57%) included less than 25 participants. Although the nature of many of these studies, particularly the intervention studies which require specialized equipment, continuous monitoring, suitable and willing participants and repeat sampling, renders them unfeasible for large scale investigations, these numbers are still strikingly small in the –omics field. Highly dimensional omic studies typically employ hundreds or thousands of subjects, and as such some of these results should be viewed with some caution, particularly as today, very few of the studies to date have attempted to directly replicate their studies in an independent cohort [76]. It should also be

noted, the vast majority of studies have been conducted in all-male or majority male populations, and the question of the role of sex in the response to PA is still to be definitively determined. The difficulty of assimilating the data from the multiple studies with vastly different designs, populations, PA/exercise context, and measures classifying and defining the activity, remains a critical challenge to understanding the impact of exercise on the metabolome. Finally, many of the metabolites identified in the PA/exercise literature have also been reported to be important in metabolomic studies of other phenotypes and many pathologies. Determining to what extent this is a reflection of these phenotype's relationship with PA/exercise, and isolating what is specific and unique to PA/exercise over other exposures is an ongoing challenge.

A further role for metabolomics, not covered here, is as an unbiased means of assessing how nutritional interventions aimed at improving PA/exercise effects and recovery may be acting at a molecular level. Metabolomic studies of nutritional supplementation and exercise, represent a fast growing sub-specialty within this field. Such studies are outside the scope of this current review, but as the evidence suggests that the metabolic changes associated with PA/exercise can be manipulated through the use of nutritional supplements [90-94], depending on their content, purpose and timing of administration, nutrition will play a crucial role in the understanding of the metabolomics of PA/exercise moving forward.

A better understanding of the differential effects of PA/exercise could help begin to explain what types of PA work best, for whom and why. It can also inform on why some people respond better to general activity versus structured training, and vice versa. Ultimately it can help to inform viable solutions for those who do not seem to benefit from PA (i.e. “non-” or “low-responders”) [95-97]. One of the biggest challenges in PA/exercise promotion is supporting long-term behavior change. It is assumed that many people do not maintain long term PA/exercise behavior as they do not enjoy it or see the hoped-for health changes in the expected timeframes. It may be that PA-metabolomics could be harnessed to identify the most effective and rewarding types of PA/exercise (aerobic, muscle strengthening, dose, and intensity) and support more long-term health behavior change.

The homogenous nature of the recommendations in PA/exercise guidelines may not be eliciting the optimum health effects across the heterogeneous populations they are aimed at. The recommendations appear to have been based on the assumption that because in an epidemiological sense the benefits of PA/exercise are so clear-cut that the physiological processes

are broadly homogeneous. This review suggests that they may not be and that in these different arenas different biological mechanisms are at work. Given the complexity of human biology (and behavior) this is hardly surprising, but its implications for public health policies around the world may be profound.

Based on our review, we have summarized some research priority areas in Table 4.

Table 4. Suggestions for Future Research Areas

Area	Recommendation
Conceptual mapping	<p>Conceptual mapping of the area of PA and metabolomics to outline key thematic areas to guide more in depth study. Themes highlighted in this current review include</p> <ul style="list-style-type: none"> • What are the long-term/chronic effects of PA/exercise? • Is there a role for sex? • How does health/disease status influence the response? • How do BMI and other lifestyle factors influence the response? • What can we learn from metabolomics profiling of different biological samples? • What can we learn through integration with other omics?
Novel human studies	<p>Generation of novel observational and intervention studies in larger more diverse populations. Based on the findings of this review this should include</p> <ul style="list-style-type: none"> • Additional studies in women • Sex stratified results where appropriate • Populations that are diverse in race and socioeconomic status • Additional studies of aerobic activity outside of running and cycling • Integrative omic studies • Discovery and validation populations
Biological mapping	<p>Construction of a biological systems map linking metabolites and metabolomics pathways with PA/exercise exposures, metabolomics responses and acute and chronic health effects</p>
Biomarker development	<p>Development of studies designed to specifically assess the utility of metabolomics biomarkers in the field of PA/exercise. As a first step, studies should move toward absolute quantification of metabolite levels. Special consideration should be given to the specificity of PA/exercise biomarkers.</p>
Expert consensus	<p>Stakeholder and consensus seeking work to describe how better understanding in this area can be harnessed to improve both health promotion, health protection and exercise training</p>

Metabolomic studies of PA/exercise help us to answer many questions, but they also raise many more that are worthy of further investigation. Accordingly, we are seeing an exponential growth of the use of metabolomics in the study of PA [17] as outlined in this review. Encouragingly, governments and research institutions are also recognizing the importance of PA-metabolomics research and are willing to make huge investments to the field, such as the United States NIH Common Fund MoTrPAC initiative [9, 10]. Thus, this field will continue to grow, and with time we may be able to answer these remaining questions and more fully understand the metabolomics response to exercise, and how this influences health and disease in populations and individuals.

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